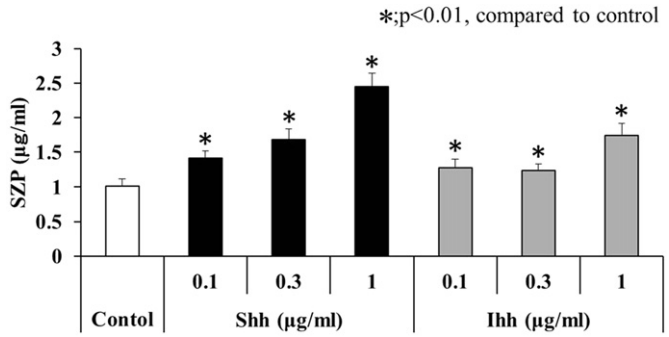

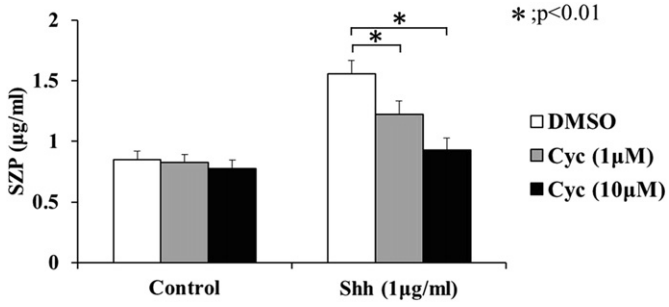

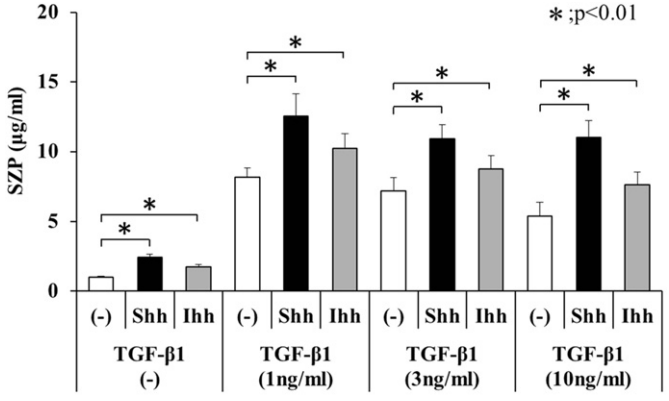

273

FUNCTIONAL ESTROGEN BIOSYNTHESIS MACHINERY IS EXPRESSED IN HUMAN POSTMENOPAUSIC OSTEOARTHRITIS CHONDROCYTES

L. Lugo, A. Villalvilla, R. Gómez, R. Largo, G. Herrero-Beaumont. *IIS Fundación Jiménez Díaz, Madrid, Spain*

Purpose: High prevalence of osteoarthritis (OA) in postmenopausic women has led to growing interest in the study of estrogen deprivation as a physiopathologic mechanism for the development of OA. In fact, ovariectomiced rabbits presented higher cartilage damage than controls. However, a relationship between circulating estrogen levels and the development of OA has not been found. Therefore local estrogens may be more important in regulating chondrocyte functions in cartilage. This data led us to investigate wether human chondrocytes can produce local estrogen despite of the systemic estrogen depletion during menopause.
Methods: Human chondrocytes were obtained from postmenopausic women (mean age 73) undergoing total knee arthroplasty. All patients were evaluated as having OA according to ACR criteria. To determine if chondrocytes produce local estradiol, cells were isolated and cultured to confluence in DMEM phenol red-free supplemented with 10% charcoal-stripped FBS and then incubated with testosterone (100ng/ml) or estrone (135ng/ml) or without any substrate (control) for 8, 24 or 48 hours. Afterwards, the presence of estradiol in chondrocytes was evaluated by immunofluorescence and additionally measured by ELISA in the culture medium. Furthermore, we assessed by semiquantitative real-time PCR the expression levels of aromatase and 17β-hydroxysteroid dehydrogenase (17βHSD) in chondrocytes after 8h and 24h of incubation with the appropriate substrate.
Results: The concentration of estradiol in the media after testosterone or estrone incubation at 8, 24 and 48 hours was higher vs control ($p<0.05$). Moreover, no significant differences were found between 24h and 48h respect 8h. The highest concentration of estradiol achieved in conditioned media was 81,3pg/ml with estrone and 68,4pg/ml with testosterone. Additionally, the immunofluorescence for estradiol in cultured cells was increased after addition of testosterone or estrone in the media vs. control. Aromatase mRNA expression was induced by testosterone after 8h, returning to basal levels at 24h. However, 17βHSD showed no induction by its substrate.
Conclusions: The findings of this study reveal that chondrocytes acting through the aromatase and 17βHSD can produce estradiol to maintain its local metabolism despite of the systemic estrogen depletion.

274

ISOTHIOCYANATES FROM THE HABITUAL DIET ARE POTENTIAL CHONDROPROTECTIVE AGENTS.

R.K. Davidson, O.J. Jupp, J. Green, R. Norton, M. O'Connell, Y. Bao, A.J. MacGregor, S.T. Donell, A. Cassidy, I.M. Clark. *Univ. of East Anglia, Norwich, United Kingdom*

Purpose: There are currently no effective disease-modifying drugs to treat OA, and drug development in this area is difficult. Our research has therefore focused on exploring the relative efficacy of a range of bioactive compounds obtained from the habitual diet with reported anti-inflammatory and/or anti-oxidant properties. We have investigated possible mechanisms by which they may be able to prevent the onset or slow the progression of OA. We have screened a number of phytochemicals (particularly organosulphur compounds (isothiocyanates and allyl sulphides) and flavonoids) for their ability to inhibit IL-1-induced metalloproteinase expression in human primary chondrocytes (HACs). Sulforaphane (SFN), an isothiocyanate which is found abundantly in broccoli, has been studied in more detail.
Methods: Several isothiocyanates (including SFN), flavonoids and other dietary compounds were screened in vitro and gene expression changes analysed by qRT-PCR. SFN was investigated further for its ability to modulate histone acetylation (a reported mechanism of SFN in other cell types, and a known regulatory mechanism for metalloproteinase expression) by Western blotting. SFN was also assayed for its ability to prevent cartilage destruction in the bovine nasal cartilage (BNC) explant model. Modulation of NFκB signalling was explored using a luciferase reporter assay, Western blotting, electrophoretic mobility assay and immunocytochemistry. Lactate dehydrogenase and caspase assays were used to test for toxicity.
Results: All ITCs tested were able to repress IL-1-induced MMP and ADAMTS mRNA expression in HACs, but differed in their potency. SFN and erysolin were the most potent, followed by iberin and erucin. Phenethyl isothiocyanate (PEITC), allyl isothiocyanate (AITC), and benzyl isothiocyanate (BITC) equally showed the least potency in IL-1-induced MMP13 and ADAMTS5 repression.
No change in histone acetylation in primary human chondrocytes (HACs) was observed in response to SFN treatment. Sulforaphane repressed interleukin-1-induced expression of extracellular matrix-degrading proteases in HACs and synoviocytes and prevented cartilage destruction in the BNC model. SFN induced the expression of Nrf2 sensitive genes e.g. haem oxygenase-1 in HACs, but knockdown of Nrf2 showed that SFN does not repress protease expression via this mechanism. SFN alters the kinetics of IκBα degradation and p65 phosphorylation. SFN inhibited NFκB translocation, DNA binding and also IL-1-induction of an NFκB sensitive promoter-reporter construct.
Conclusions: Isothiocyanates represent a group of phytochemicals with potential chondroprotective properties. SFN can dose-dependently attenuate the induction of metalloproteinase expression and protect against